A Highly Sensitive Enzyme-Linked Immunosorbent Assay for the Detection of Circulating Anti-BP180 Autoantibodies in Patients with Bullous Pemphigoid

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The BP180 antigen, a component of the epidermal anchoring complex, has been identified as one of the major antigenic targets of autoantibodies associated with the blistering skin disease, bullous pemphigoid. Our research group has recently demonstrated that reactivity of bullous pemphigoid autoantibodies to the BP180 ectodomain is almost entirely restricted to a set of four antigenic sites clustered within the membrane-proximal noncollagenous stretch (NC16A). Using a passive transfer mouse model, antibodies to the corresponding noncollagenous region of murine BP180 were shown to trigger an inflammatory subepidermal blistering disease that closely mimics bullous pemphigoid. We now report the development of an enzyme-linked immunoabsorbent assay system that is extremely sensitive in detecting disease-specific autoantibodies in the sera of bullous pemphigoid patients. The target antigen in this assay is a recombinant form of the BP180 NC16A domain that contains all four of the well-defined bullous pemphigoid-associated antigenic sites. Of 50 randomly selected bullous pemphigoid sera tested, 47 (94%) were positive in this assay, whereas no specific reactivity was detected in any of the 107 controls. Interestingly, all three of the bullous pemphigoid sera that were negative in this assay had been obtained from patients who were already undergoing treatment. The NC16A enzyme-linked immunosorbent assay is more sensitive than any of the standard techniques for detecting circulating bullous pemphigoid autoantibodies, including other enzyme-linked immunosorbent assays, immunoblotting, and indirect immunofluorescence. Finally, the NC16A enzyme-linked immunosorbent assay provides immunologic information that cannot be obtained from direct immunofluorescence studies of skin biopsies, and that may well be relevant in the diagnosis and treatment of bullous pemphigoid. ***Key words: autoantigen/collagen/epitope/hemidesmosome. J Invest Dermatol 109:679–683, 1997***

Bullous pemphigoid (BP) is a subepidermal autoimmune blistering disease first described by Lever (1953). This disease primarily affects the elderly and is characterized by the linear deposition of IgG and C3 at the cutaneous basement membrane zone (BMZ) (Jordon *et al*, 1967). Autoantibodies in BP are directed to two proteins associated with the anchoring complex, BP230 and BP180, also known as BPAG1 and BPAG2, respectively (Stanley *et al*, 1981; Labib *et al*, 1986; Stanley *et al*, 1988; Mueller *et al*, 1989; Diaz *et al*, 1990; Giudice *et al*, 1992). BP230 localizes to the intracellular hemidesmosomal plaque (Tanaka *et al*, 1991), whereas BP180 is a transmembrane glycoprotein whose extracellular domain consists of 15 interrupted collagen domains (Giudice *et al*, 1992; Li *et al*, 1993). In addition to BP, autoantibodies to BP180 have been found to be associated with two other subepithelial blistering diseases, herpes gestationis and cicatricial pemphigoid (Giudice *et al*, 1993; Balding *et al*, 1996). The pathogenic relevance of anti-BP180 antibodies has been established using a passive transfer mouse model (Liu *et al*, 1993, 1995). Rabbits were immunized with a recombinant form of murine BP180 and the purified rabbit IgG was injected into neonatal mice. The antibody-treated mice developed a subepidermal blistering disease that duplicated the key features of BP, i.e., IgG and C3 deposition at the BMZ, inflammatory infiltration of the upper dermis, and detachment of the epidermis from the basement membrane.

Epitope mapping studies of human BP180 have shown that the NC16A domain of this protein harbors all of the major extracellular antigenic sites recognized by BP sera (Giudice *et al*, 1993; Zillikens *et al*, 1997). Immunoreactivity of BP sera to the BP180 ectodomain can be abolished by depletion of autoantibodies to the BP180 NC16A domain. It was further shown that NC16A contains four distinct antigenic sites recognized by BP autoantibodies. Interestingly, all four sites are clustered within the *N*-terminal 45 amino acid stretch of this noncollagenous extracellular domain. By immunoblotting, 15 of 15 BP sera reacted with a bacterial fusion protein containing this 45 amino acid stretch of NC16A (Zillikens *et al*, 1997).

A previous enzyme-linked immunosorbent assay (ELISA) for BP180 autoantibodies used a recombinant form of NC16A consisting of

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Abbreviations: BMZ, basement membrane zone; BP, bullous pemphigoid; BSA, bovine serum albumin; EBA, epidermolysis bullosa acquisita; ELISA, enzyme-linked immunosorbent assay; FS, fogo selvagem; GST, glutathione S-transferase; IF, immunofluorescence; OD, optical density; PBS, phosphate-buffered saline; PV, pemphigus vulgaris; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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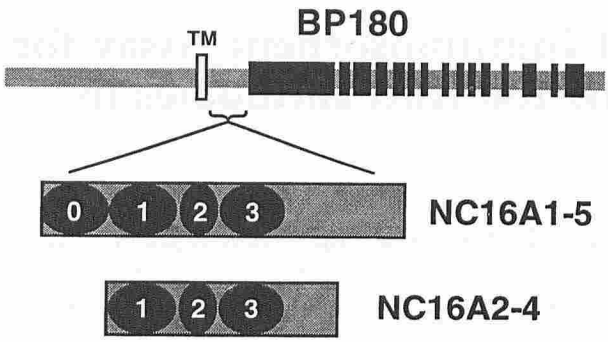

Figure 1. Schematic diagram showing the segments of human BP180 present in the recombinant proteins GST-NC16A1–5 (used in the present ELISA) and GST-NC16A2–4 (used in a previous ELISA). TM, transmembrane domain; n, extracellular collagenous domains of the human BP180 protein. Ovals labeled "0," "1," "2," and "3" represent the four epitopes that have been identified within the *N*-terminal 45 amino acids of the BP180 NC16A domain (Zillikens *et al*, 1997). The entire NC16A domain is present in the GST-NC16A1–5 fusion protein, whereas GST-NC16A2–4, used in a previously reported ELISA (Giudice *et al*, 1994), contains only three of the four well-characterized BP-associated epitopes.

42 amino acids (Giudice *et al*, 1994). The assay detected BP180 autoantibodies in 53% of BP sera. The recombinant protein used in this ELISA lacked the *N*-terminal 17 amino acids of NC16A, a stretch containing one of the recently defined antigenic sites, and one that is recognized by 60% of BP sera by immunoblotting (Zillikens *et al*, 1997). The aim of the current study was to develop a highly sensitive ELISA for the detection of autoantibodies to BP180 using a recombinant form of full-length NC16A, which encompasses all four of the well-defined antigenic sites associated with BP.

MATERIALS AND METHODS

Patients and sera Serum samples were obtained from 50 well-characterized BP patients. In 22 cases, the sample was obtained before treatment was initiated, whereas 12 patients were under therapy at the time of the blood draw. Information on treatment was not available in the remaining cases. All BP patients had linear deposits of C3 and/or IgG at the BMZ in perilesional skin biopsies by direct immunofluorescence (IF) assays. By indirect IF analysis on 1 M NaCl-separated human skin (Gammon *et al*, 1984; Zillikens *et al*, 1996), 42 BP sera showed IgG reactivity with the epidermal side of the split, whereas the remaining eight were negative. Controls (n = 107) included sera from patients with epidermolysis bullosa acquisita (EBA; n = 12), pemphigus vulgaris (PV; n = 18), the Brazilian endemic form of pemphigus foliaceus [also known as fogo selvagem (FS; n = 30)], and healthy volunteers (n = 47). All EBA sera had circulating autoantibodies binding exclusively to the dermal side of the split, and 10 of the 12 EBA sera reacted with the 290-kDa EBA autoantigen by immunoblotting. All PV and FS sera yielded the typical intercellular staining pattern by indirect IF on monkey esophagus and human neonatal foreskin, respectively. All 15 of the PV sera that were tested by immunoprecipitation showed reactivity with the extracellular domain of desmoglein 3. All FS sera immunoprecipitated bovine desmoglein 1 as described previously (Olague-Alcala *et al*, 1994).

Preparation of recombinant proteins A DNA segment encoding the entire NC16A domain was obtained by polymerase chain reaction amplification using a previously cloned human BP180 cDNA as template (Giudice *et al*, 1992; Zillikens *et al*, 1997). The amplification product was subcloned into pGEX-1T (Pharmacia, Piscataway, NJ) using an engineered NotI site. The resulting glutathione S-transferase (GST)-NC16A1–5 fusion protein and recombinant GST were expressed in *Escherichia coli* strain DH5α and purified by glutathione-agarose affinity chromatography, as described previously (Giudice *et al*, 1993). The recombinant form of NC16A used in this ELISA is shown in **Fig 1**.

Immunoblotting of recombinant proteins and skin extracts Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed as described (Giudice *et al*, 1993). Affinity purified recombinant proteins were fractionated by 15% SDS-PAGE. Epidermal and dermal extracts were prepared as described (Labib *et al*, 1986; Bernard *et al*, 1990), and fractionated by 6% and 5% SDS-PAGE, respectively. Sera of patients and controls were preadsorbed with a bacterial cell lysate containing recombinant GST to eliminate reactivity with the GST moiety of the fusion protein and used at a 100-fold dilution. Immunoadsorptions were verified to be complete by the absence of reactivity with recombinant GST.

ELISA protocol In the ELISA used in this study, immobilized GST-NC16A1–5 was probed with a series of BP and control sera using a horseradish peroxidase-conjugated secondary antibody. As a background control, the same sera were tested for reactivity with a molar equivalent amount of GST. The entire assay was performed at room temperature on 96-well microtiter plates (Immulon 4, Dynatech, Chantilly, VA).

Defining the optimal working conditions of the assay followed established guidelines (Kemeny, 1991; Crowther, 1995). Chessboard titrations were performed with serial dilutions of antigen (3.2 μg to 3.1 ng) and secondary antibody (500–64,000-fold dilutions) and a constant 100-fold dilution of a primary antibody (a BP serum with medium range reactivity to BP180). Analysis of the curves generated by plotting antigen concentration *versus* OD_{492} revealed that a 10,000-fold dilution of the secondary antibody produced optimal discrimination in this assay. Lower dilutions of secondary antibody resulted in an increase in background labeling, higher dilutions resulted in a loss of sensitivity. In a similar fashion, chessboard titrations were performed with serial dilutions of antigen (3.2 μg to 3.1 ng) and primary antibody (25–3200-fold dilutions of a BP serum) and a constant 10,000-fold dilution of the secondary antibody. Analysis of the plot of antigen concentration *versus* primary antibody dilution revealed that a 200-fold dilution of the primary antibody yielded the highest level of discrimination. The data also showed that maximal levels of target antigen were adsorbed when 400 ng of GST-NC16A1–5 were applied to each well. Coating of wells at 4°C overnight did not noticeably alter the amount of antigen adsorbed.

The optimized ELISA was run under the following conditions. Each well was coated for 2 h with 400 ng of purified recombinant GST-NC16A1–5 in 50 μl of 0.1 M bicarbonate buffer, pH 9.6. Wells were then washed five times with phosphate-buffered saline (PBS), pH 7.2, using an automated microplate washer (model 1550, Biorad, Richmond, CA), and blocked for 1 h with 100 μl PBS containing 1% bovine serum albumin (BSA; Sigma, St. Louis, MO). Subsequently, wells were incubated for 1 h with 50 μl of a primary serum (BP or control) diluted 200-fold in PBS containing 1% BSA and 0.05% Tween-20. After washing as above, each well was incubated for 1 h with 50 μl horseradish peroxidase-labeled goat anti-human IgG (Kirkegaard and Perry, Gaithersburg, MD) diluted 10,000-fold in PBS with 1% BSA and 0.05% Tween-20. Following another series of washes, 50 μl of substrate solution (o-phenylenediamine in 0.1% H_2O_2) were added to each well. Ten min after substrate addition, the reaction was stopped by the addition of 50 μl 2N H_2SO_4, and the OD_{492} was measured using a microplate reader (model 2550; Biorad). Each serum was assayed in triplicate for reactivity with both GST-NC16A1–5 and recombinant GST. For each serum tested, the mean optical density (OD) reading obtained with GST was subtracted from the mean reading obtained with GST-NC16A1–5.

In order to minimize plate-to-plate variability, the ELISA value of each serum on a plate was adjusted relative to a standard curve generated from the ELISA results of one negative reference serum and three serial dilutions of a BP-positive reference serum. A two piece linear spline (Ertel and Folkes, 1976) was fit to the data to provide this adjustment. As applied here, the piecewise linear spline is the best least squares fit of two straight line segments to the reference data for all plates. Use of such a spline provided a slightly better fit to the reference data over using a single straight line. The join point (knot) of the spline was chosen to be 0.612 OD units in order to maximize the precision of the standard curve over the range of 0–0.612 OD units, a range that encompasses all of the control data and the lowest readings from the BP group. Based on the results from the internal standards, OD readings showed a linear response up to a value of 1.5 OD units. Sera that yielded values higher than 1.5 OD units were retested at higher dilutions until readings within the linear range were obtained.

RESULTS

The NC16A ELISA is a highly sensitive technique for detecting anti-BP180 autoantibodies in the sera of BP patients A large number of BP and control sera were tested for reactivity with the BP180 NC16A domain using the above-described ELISA protocol. To establish the background cut-off for this assay, a statistical analysis was performed on the NC16A ELISA results obtained with the four control groups (EBA, FS, PV, and normal human sera). A one-way analysis of variance showed no significant difference in the mean OD_{492} readings obtained from these four groups ($p = 0.18$). According to the Ryan–Joiner test for normality (Shapiro and Wilk, 1963), OD_{492} readings from the four individual control groups, as well as the pooled data from these groups, were consistent with a Gaussian distribution

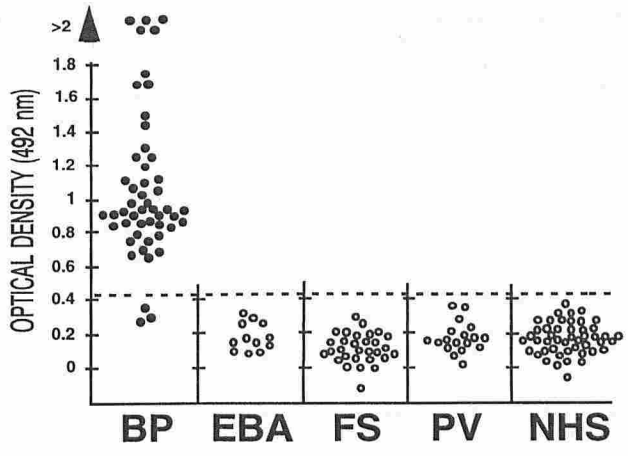

Figure 2. The NC16A ELISA is a specific and sensitive tool for the diagnosis of BP. BP and control sera [from patients with epidermolysis bullosa acquisita (EBA), pemphigus vulgaris (PV), fogo selvagem (FS), and healthy controls (NHS)] were incubated at a 200-fold dilution with immobilized NC16A-GST fusion protein and recombinant GST. Each serum was tested in triplicate with both proteins and the plotted points represent the difference of the means of the OD_{492} readings obtained with GST-NC16A1–5 and recombinant GST. ·····, cut-off of the assay (mean OD_{492} of pooled controls + 3SD).

($p = 0.40$ for the pooled data). The raw data from each plate were adjusted using a linear spline as described in *Materials and Methods*. The mean of the adjusted data from the 107 control sera was 0.168 with a standard deviation of 0.089. The cut-off for the NC16A ELISA was set at 0.443 OD units (a z-value of 3.08), which yielded an observed specificity of 100% (none of the 107 controls exceeded this value) and a theoretical specificity of 99.9% (i.e., based on the observation that the control data are normally distributed, one control serum in 1000 is predicted to exceed this cut-off). Variability of the results of this assay was established by calculating the coefficient of variance exhibited by an internal reference BP serum with an intermediate OD reading. The unadjusted OD readings showed a 15.3% variance between runs and a 10.7% variance between plates on a given run. After adjustment of the data based on the internal standard curves, the coefficients of variance were reduced to 3.9% and 1.0% for the run-to-run and plate-to-plate comparisons, respectively.

As shown in **Fig 2**, 47 of the 50 BP sera included in this study (94%) exhibited specific reactivity with NC16A. Any sera that yielded OD_{492} readings higher than 1.5 at the standard 200-fold dilution were diluted further to obtain readings within the linear range of the plate reader. Twenty-two of the 50 BP sera were obtained during the early stage of the disease before any treatment was initiated. In all of these sera, BP180 autoantibodies were detected by ELISA. Of the 12 sera that were collected during therapy, nine samples contained anti-BP180 antibodies, and three sera had readings below the cut-off. Two of the ELISA-negative sera were from patients who had been undergoing treatment with oral corticosteroids for 3 and 7 mo. The third BP patient, whose serum fell below the ELISA cut-off, had been treated with tetracycline and niacinamide for 8 mo. All three patients still had skin lesions at the time serum samples were obtained. For 16 of the BP sera included in this study, no information was available as to whether the blood had been drawn before or during treatment. All 16 sera in this last group, however, were positive by ELISA.

Detection of circulating BP autoantibodies by the NC16A ELISA is more sensitive than immunoblotting or indirect immunofluorescence techniques Forty-five of the 50 BP sera in this study (90%) showed specific reactivity with the GST-NC16A1–5 fusion protein by immunoblotting. Three of the five BP sera that failed to react with NC16A by immunoblotting were positive in the NC16A ELISA. On the other hand, one BP serum that fell below the cut-off in the NC16A ELISA showed specific reactivity with NC16A by immunoblotting (**Table I**).

When tested by immunoblotting against a human epidermal extract, 29 of the 50 BP sera (58%) reacted with BP180, and all of these BP180-positive sera were also positive in the NC16A ELISA. Thirty of the 50 BP sera (60%) contained autoantibody reactivity to the BP230 antigen, as determined by immunoblotting using a human epidermal extract. Interestingly, all three of the BP sera that were negative in the NC16A ELISA did react with BP230 by immunoblotting. Representative immunoblot labeling patterns for BP sera are shown in **Fig 3**. The immunoblots in the left and middle panels of this figure show reactivity with GST-NC16A1–5 (*lanes 2* and *4*), but only the blot on the left shows reactivity with epidermal BP180 (*lane 1*). The serum that was used to label the immunoblot on the right reacts with epidermal BP230 (*lane 5*), but does not recognize epidermal BP180 (*lane 5*) or the GST-NC16A1–5 fusion protein (*lane 6*).

By indirect IF on salt-split skin, 42 of the 50 BP sera (84%) contained autoantibodies binding to the epidermal side of the split. Immunoblotting revealed that, of the 42 sera positive by indirect IF, 28 reacted with epidermal BP230, 25 reacted with BP180 from an epidermal extract, and 37 reacted with the GST-NC16A1–5 fusion protein by immunoblotting. Also among the BP sera that showed an epidermal-side IF labeling pattern on salt-split skin were two that had no detectable anti-BP180 autoantibodies, as determined by the NC16A ELISA or immunoblotting with either recombinant or tissue-derived BP180. Both of these BP180-negative sera contained autoantibodies that recognized BP230 by immunoblotting, and both serum samples had been obtained while the patients were undergoing therapy. Among the eight sera that exhibited no labeling by indirect IF at a dilution of 1:20 were two that reacted with BP230 and four that reacted with BP180 by immunoblotting with an epidermal extract. All eight of the indirect IF-negative BP sera reacted with GST-NC16A1–5 by immunoblotting, and seven were positive in the NC16A ELISA.

DISCUSSION

In this paper, we report the development and characterization of a highly specific and sensitive ELISA for the detection of autoantibodies to BP180 in the sera of patients with BP. The recombinant form of BP180 that was used in this study contained all of the major extracellular epitopes recognized by autoantibodies in BP sera, as defined in a recent epitope mapping study by our group (Zillikens *et al*, 1997). In this ELISA system, autoantibodies to the BP180 NC16A domain were detectable in 47 of the 50 BP sera tested. The overall sensitivity of the assay was 94%, at a calculated specificity of 99.9%.

Other previously reported ELISA systems designed to detect circulating BP autoantibodies show much lower levels of sensitivity compared with the present NC16A ELISA. Ide *et al* (1995) developed an ELISA using recombinant forms of the *C*-terminal domain of murine BP230. Approximately 50% of BP sera reacted with these recombinant proteins by immunoblotting, and most of the immunoblot-positive sera also recognized the proteins by ELISA. Another recent ELISA study reports a sensitivity of 62% for the detection of IgE antibodies to a recombinant form of BP230 (Delaporte *et al*, 1996). Our laboratory previously described an ELISA in which 53% of BP sera showed reactivity with a recombinant form of BP180 (Giudice *et al*, 1994). The recombinant protein used in this previous assay (designated GST-SΔ1 or GST-NC16A2–4) contained a 42 amino acid segment of NC16A, but lacked the *N*-terminal 17 amino acids of this BP180 domain. In a recent epitope mapping study, this 17 amino acid stretch of the BP180 ectodomain was found to contain an antigenic site recognized by 60% of BP sera (Zillikens *et al*, 1997). Of 15 BP sera tested, two reacted with this stretch and no other site within NC16A. Inclusion or exclusion of this short stretch of BP180 may well account for at least part of the large difference in sensitivity levels exhibited by the NC16A and the NC16A2–4 ELISA protocols (94% *vs* 53%). The enhanced sensitivity and specificity of the present ELISA can also be attributed to several other alterations made in the parameters of the assay that were based on a rigorous and systematic optimization strategy, including chessboard titrations of serial antigen concentrations, serum dilutions, and conjugate dilutions.

Compared with direct IF analysis of skin biopsies from patients (for

Table I. Detection of autoantibodies in BP sera by the NC16A ELISA is more sensitive than immunoblotting and indirect IF

	Immunoblotting *versus* GST-NC16A1–5[a]		Immunoblotting *versus* human epidermal extract[b]				Indirect IF labeling of salt-split skin[c]		
			Reactivity with BP180		Reactivity with BP230				
	Pos	Neg	Pos	Neg	Pos	Neg	Epidermal side	Dermal side	Neg
NC16A ELISA Positive (n = 47)	44	3	29	18	27	20	40	0	7
NC16A ELISA Negative (n = 3)	1	2	0	3	3	0	2	0	1
TOTAL	45	5	29	21	30	20	42	0	8

[a]BP sera (diluted 100-fold and preadsorbed against recombinant GST) were tested for reactivity with affinity purified GST-NC16A1–5 on immunoblots.
[b]BP sera were used at a 100-fold dilution.
[c]1 M NaCl-separated human skin was incubated with a 1:20 dilution of patients' sera, followed by an incubation with fluoroscein isothiocyanate-labeled goat anti-human IgG.

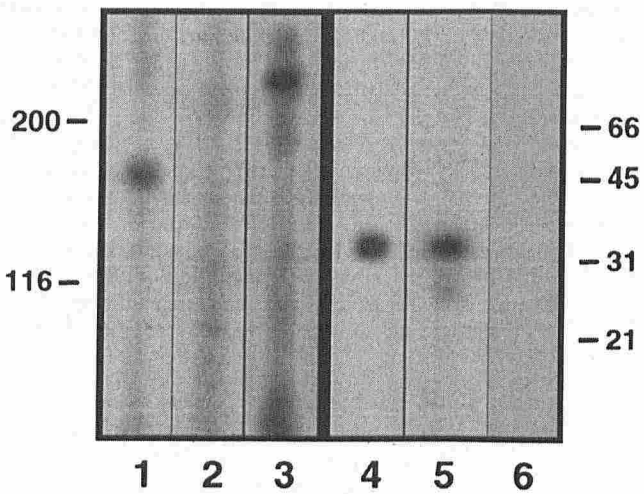

Figure 3. Three representative immunoblot labeling patterns exhibited by BP sera. Immunoblots containing an epidermal protein extract (*lanes 1–3*) and recombinant GST-NC16A1–5 (*lanes 4–6*) were labeled with three BP sera. One BP serum reacted with both the epidermal-derived and the recombinant form of BP180 (*lanes 1* and *4*, respectively). The second BP serum showed no specific reactivity on the epidermal blot (*lane 2*), but did react with GST-NC16A1–5 (*lane 5*). The third BP serum reacted with epidermal BP230 (*lane 3*) and did not recognize BP180 from either the epidermal extract (*lane 3*) or the GST-NC16A1–5 preparation (*lane 6*). The two BP sera that reacted with GST-NC16A1–5 by immunoblotting (*lanes 4* and *5*) were also positive in the NC16A ELISA, whereas the third BP serum was negative in the ELISA. The immunoblot labeling patterns shown here are representative of those obtained with the BP sera in this study. In this immunoblot assay, reactivity with GST was eliminated by preadsorption of the BP sera with recombinant GST. Shown to the left are the positions of the molecular weight markers (in kDa) for a 6% polyacrylamide gel (*lanes 1*, *2*, and *3*), whereas the markers on the right (also in kDa) are for a 15% gel (*lanes 4*, *5*, and *6*).

detection of tissue bound immunoreactants), the NC16A ELISA is less invasive (requiring only a blood draw) and provides additional immunologic information. All untreated BP patients (22 of 22) were positive in both of these assays. Three of the 50 BP sera in this study were negative in the NC16A ELISA, but were positive by direct IF, suggesting that direct IF is the more sensitive assay. It should be kept in mind, however, that these three ELISA-negative sera were obtained from BP patients well after treatment had begun. It might turn out, after further investigation, that reactivity with NC16A is a specific marker for the initial stages of BP, and if so, the NC16A ELISA might prove to be more useful than direct IF analysis for monitoring the course of the disease.

The BP180 NC16A ELISA was found to be more sensitive than any of the other standard techniques for detecting circulating autoantibodies in BP. Other methods that are known to have a high sensitivity in detecting BP autoantibodies include indirect IF on salt-split skin and immunoblotting using either an epidermal extract or recombinant BP180. The NC16A ELISA detected anti-BP180 autoantibodies in seven of the eight BP sera that were negative by indirect IF and in all 20 BP sera that failed to react with BP230 by immunoblotting. Conversely, all three BP sera that were negative in the NC16A ELISA showed reactivity with BP230 by immunoblotting, and two of these three sera were positive by indirect IF. These findings corroborate previous reports that certain BP sera react with BP230, but not with BP180. It is noteworthy that the three sera that fell into this category were from BP patients who were undergoing therapy for their disease at the time the blood samples were obtained. These findings are consistent with the hypothesis that the early stages of BP are characterized by an anti-BP180 autoimmune response, whereas an autoantibody response directed against BP230 may predominate in the later stages of disease. Experiments designed to test this hypothesis are currently under way.

In this study, we found that 90% of BP sera (45 of 50) reacted with the GST-NC16A1–5 fusion protein by immunoblotting, a result that is in agreement with data from a similar experimental set-up reported by Matsumura *et al* (1996). Three BP sera from our study that failed to react with this fusion protein by immunoblotting were positive in the NC16A ELISA, suggesting that the ELISA is the more sensitive of these two techniques. There was, however, one BP serum that did react with GST-NC16A1–5 on an immunoblot, but fell below the cut-off in the NC16A ELISA. It is possible that antibodies in the serum of this patient react with a denaturation-dependent epitope located in the NC16A domain.

In conclusion, we have developed an ELISA system that provides a specific, sensitive, and rapid method for the detection of circulating anti-BP180 autoantibodies in BP. Further studies will test the usefulness of this assay for the diagnosis of other bullous dermatoses characterized by autoantibodies to BP180, including herpes gestationis and cicatricial pemphigoid.

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